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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: Bettina MOECKEL et al

SERIAL NO: 10/075,460 GROUP ART UNIT: 1645

FILED: February 15, 2002

FOR: NUCLEOTIDE SEQUENCES WHICH CODE FOR THE RPSL GENE

INFORMATION DISCLOSURE STATEMENT UNDER 37 C.F.R. §1.97

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SIR:

Applicants wish to disclose the following information.

REFERENCES

☐ The Applicants wish to make of record the references listed on the attached Form PTO-1449. Copies of the listed references are attached, where required, as are either statements of relevancy or any readily available English translations of pertinent portions of any non-English language references.

☐ A check is attached in the amount required under 37 C.F.R. §1.17(p).

RELATED CASES

■ Attached is a list of Applicants' pending applications which may be related to the present application. A copy of the claims and drawings of the pending applications is attached along with Form PTO-1449.

☐ A check is attached in the amount required under 37 C.F.R. §1.17(p).

CERTIFICATION

☐ Each item of information contained in this Information Disclosure Statement was first cited in a communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of this statement.

☐ No item of information contained in this Information Disclosure Statement was cited in a communication from a foreign patent office in a counterpart foreign application or, to the knowledge of the undersigned, having made reasonable inquiry, was known to any individual designated in 37 CFR §1.56(c) more than three months prior to the filing of this statement.

DEPOSIT ACCOUNT

■ Please charge any additional fees for the papers being filed herewith and for which no check is enclosed herewith, or credit any overpayment to deposit account number 15-0030. A duplicate copy of this sheet is enclosed.

Respectfully submitted,

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LIST OF RELATED CASES

Docket Number	Serial or Patent Number	Filing or Issue Date	Inventor/ Applicant
218472US0 X*	10/075,460	02/15/02	MOECKEL, et al.
222970US0 X	10/156,856	05/30/02	BATHE, et al.
229008US0 X	10/270,512	10/16/02	BATHE, et al.

*Present Application; listed for information

WHAT IS CLAIMED IS:

1. An isolated polynucleotide sequence, which encodes a polypeptide having the amino acid sequence of SEQ ID NO. 2.

2. The isolated polynucleotide sequence of Claim 1, wherein said polypeptide sequence has *MetD* transcription regulator activity.

3. A vector comprising the isolated polynucleotide sequence of Claim 1.

4. A host cell comprising the isolated polynucleotide sequence of Claim 1.

5. The host cell of Claim 4, which is a *Coryneform* bacterium.

6. The host cell of Claim 4, which is a *Coryneform* bacterium selected from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium acetoglutamicum*, *Corynebacterium acetoacidophilum*, *Corynebacterium melassecola*, *Corynebacterium thermoaminogenes*, *Brevibacterium flavum*, *Brevibacterium lactofermentum*, and *Brevibacterium divaricatum.*

7. A method for detecting polynucleotides with at least 70% homology to the polynucleotide of Claim 1, comprising contacting a polynucleotide sample with a polynucleotide comprising at least 15 consecutive nucleotides of the polynucleotide sequence of Claim 1, or at least 15 consecutive nucleotides of the complement thereof.

8. A method for producing polynucleotides with at least 70% homology to the polynucleotide of Claim 1, comprising contacting a polynucleotide sample with a polynucleotide comprising at least 15 consecutive nucleotides of the polynucleotide sequence of Claim 1, or at least 15 consecutive nucleotides of the complement thereof.

9. A process for screening for polynucleotide sequences, which encode a polypeptide having *MetD* transcription regulator activity comprising

(a) hybridizing the isolated polynucleotide to Claim 1 to a polynucleotide sample to be screened;

(b) expressing the polynucleotide to produce a polypeptide;

(c) detecting the presence or absence of *MetD* transcription regulator activity of the polypeptide.

10. The process according to Claim 9, wherein said hybridizing is performed with arrays, micro arrays, DNA chips, or combinations thereof.

11. A method for making *MetD* transcription regulator polypeptide, comprising

(a) culturing the host cell of Claim 4 for a duration of time under conditions suitable for expression of *MetD* transcription regulator polypeptide; and

(b) collecting the *MetD* transcription regulator polypeptide.

12. An isolated polynucleotide, which comprises SEQ ID NO. 1.

13. An isolated polynucleotide, which is complementary to the polynucleotide of Claim 12.

14. An isolated polynucleotide, which is at least 70% identical to the polynucleotide of Claim 12.

15. An isolated polynucleotide, which is at least 80% identical to the polynucleotide of Claim 12.

16. An isolated polynucleotide, which is at least 90% identical to the polynucleotide of Claim 12.

17. An isolated polynucleotide, which comprises at least 15 consecutive nucleotides of the polynucleotide of Claim 12.

18. An isolated polynucleotide, which hybridizes to the polynucleotide of Claim 12.

19. The isolated polynucleotide of Claim 12, which encodes a polypeptide having *MetD* transcription regulator activity.

20. A vector comprising the isolated polynucleotide of Claim 12.

21. A host cell comprising the isolated polynucleotide of Claim 12.

22. The host cell of Claim 21, which is a *Coryneform* bacterium.

23. The host cell of Claim 21, which is a *Coryneform* bacterium selected from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium acetoglutamicum*, *Corynebacterium acetoacidophilum*, *Corynebacterium melassecola*, *Corynebacterium thermoaminogenes*, *Brevibacterium flavum*, *Brevibacterium lactofermentum*, and *Brevibacterium divaricatum*.

24. A process for screening for polynucleotide sequences, which encode a polypeptide having *MetD* transcription regulator activity comprising

(a) hybridizing the isolated polynucleotide to Claim 11 to a polynucleotide sample to be screened;

(b) expressing the polynucleotide to produce a polypeptide;

(c) detecting the presence or absence of *MetD* transcription regulator activity of the polypeptide.

25. A method for detecting polynucleotides with at least 70% homology to the polynucleotide of Claim 12, comprising contacting a polynucleotide sample with a polynucleotide comprising at least 15 consecutive nucleotides of the polynucleotide sequence of Claim 12, or at least 15 consecutive nucleotides of the complement thereof.

26. A method for producing polynucleotides with at least 70% homology to the polynucleotide of Claim 12, comprising contacting a polynucleotide sample with a polynucleotide comprising at least 15 consecutive nucleotides of the polynucleotide sequence of Claim 12, or at least 15 consecutive nucleotides of the complement thereof.

27. A method for making *MetD* transcription regulator polypeptide, comprising

(a) culturing the host cell of Claim 21 for a duration of time under conditions suitable for expression of *MetD* transcription regulator polypeptide; and

(b) collecting the *MetD* transcription regulator polypeptide.

28. A *Coryneform* bacterium, which comprises attenuated expression of the *metD* gene.

29. The *Coryneform* bacterium of Claim 28, wherein the *metD* gene comprises the polynucleotide sequence of SEQ ID NO. 1.

30. *Corynebacterium glutamicum* strain ATCC13032delta*metD* .

31. *Escherichia coli* DH5αmcr/pK18mobsacB*metD* del.

32. A process for producing L-amino acids comprising culturing a bacterial cell in a medium suitable for producing L-amino acids, wherein the bacterial cell comprises attenuated expression of the *metD* gene.

33. The process of claim 32, wherein said bacterial cell is a *Coryneform* bacterium.

34. The process of claim 33, wherein the bacterial cell is a *Coryneform* bacterium from the group consisting of *Corynebacterium glutamicum*, *Corynebacterium acetoglutamicum*, *Corynebacterium acetoacidophilum*, *Corynebacterium melassecola*, *Corynebacterium thermoaminogenes*, *Brevibacterium flavum*, *Brevibacterium lactofermentum*, and *Brevibacterium divaricatum*.

35. The process of Claim 32, wherein the *metD* gene comprises the polynucleotide sequence of SEQ ID NO. 1.

36. The process of Claim 32, wherein the L-amino acid is L-methionine.

37. The process of Claim 32, wherein the bacteria further comprises at least one gene whose expression is enhanced, wherein the gene is selected from the group consisting of the *gap*, *tpi*, *pgk*, *zwf*, *pyc*, *lysC*, *hom*, *metA*, *metB*, *aecD*, *metY*, and *glyA*.

38. The process of Claim 32, wherein the bacteria further comprises at least one gene

whose expression is attenuated, wherein the gene is selected from the group consisting of the *pck*, *pgi*, *poxB*, *thrB*, *thrC*, *metK*, and *ddh*.

39. The process of Claim 32, wherein the bacterial cell is *Corynebacterium glutamicum* strain ATCC13032delta*metD* .

40. An isolated polypeptide comprising the sequence of SEQ ID NO. 2.

41. An isolated polypeptide comprising an amino acid sequence, which is at least 70% identical to the peptide of Claim 40.

42. A process for the preparation of an animal food additive, comprising

a) culturing at least one L-methionine-producing microorganism in a fermentation medium;

b) removing water from the fermentation medium;

c) removing from 0 to 100 wt.% of the biomass from the fermentation medium formed during the culturing; and

d) drying the fermentation medium.

43. The process according to Claim 42, wherein the at least one microorganism comprises genes of the biosynthesis pathway of L-methionine are that are enhanced.

44. The process according to Claim 42, wherein the at least one microorganism comprises genes of the biosynthesis pathway of L-methionine are that are attenuated.

45. The process according to Claim 42, wherein the at least one microorganism comprises a polynucleotide which encodes an *metD* gene, wherein the *metD* gene is attenuated.

46. The process according to Claim 42, wherein the at least one microorganism is *Corynebacterium glutamicum*.

47. The process according to Claim 42, wherein the at least one microorganism is

Corynebacterium glutamicum strain ATCC13032delta*metD* .

48. The process according to Claim 42, further comprising at least one of the following steps:

e) adding at least one organic substance selected from the group consisting of L-methionine and D-methionine to the fermentation medium ;

f) adding at least one auxiliary substance selected from the group consisting of silicas, silicates, stearates, grits, and bran to the fermentation medium; or

g) converting the fermentation medium obtained from at least one step selected from the group consisting a), b), c), d), e), and f) into an animal food additive.

49. The process according to Claim 48, wherein the converting is performed by coating the fermentation medium with at least one film-forming agent.

50. An animal food additive made by the process according to Claim 49, wherein the animal food additive is stable in the stomach of an animal.

51. An animal food additive made by the process according to Claim 49, wherein the animal food additive is stable in the rumen of an animal.

52. An animal food additive made by the process according to Claim 49, wherein the at least one film-forming agent is selected from the group consisting of metal carbonates, silicas, silicates, alginates, stearates, starches, gums, and cellulose ethers.

53. An animal food additive made by the process according to Claim 42, wherein the food additive comprises at most 5 wt % of water.

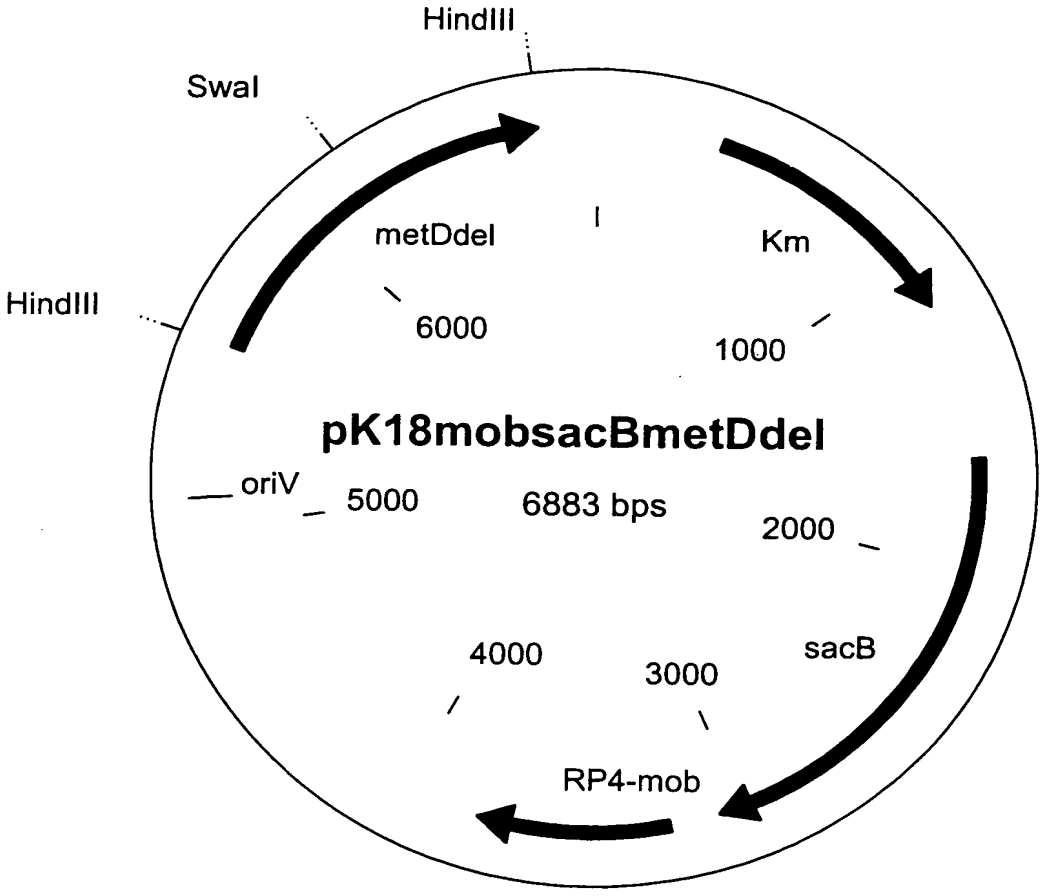
54. An animal food additive made by the process according to Claim 42, wherein the food additive comprises at most 2 wt % of water.

55. An animal food additive made by the process according to Claim 42, wherein the food additive comprises from 1 wt.% to 80 wt.% L-methionine, D-methionine, D,L methionine, or a mixture thereof based on the dry weight of the animal feedstuffs additive; and from 1 to 40 wt.% L-lysine, D-lysine, D,L-lysine, or a mixture thereof based on the dry weight of the animal feedstuffs additive.

ABSTRACT

The invention relates to polynucleotides comprising polynucleotide sequences corresponding to the *metD* gene and parts thereof that encode polypeptide sequences and parts thereof possessing varying degrees of *MetD* transcription regulator activity, methods for preparation of L-amino acids, and methods of screening and amplifying polynucleotides encoding polypeptide sequences which comprise varying degrees of *MetD* transcription regulator activity. Further, the invention relates to animal food additives based on fermentation liquor and containing L-methionine, and to the preparation of such additive.

Figure 1: Map of the plasmid pK18mobsacBmetDdel



CLAIMS:

1. A replicatable nucleic acid originating from corynebacteria and coding for an aspartokinase, wherein the amino acid sequence of the aspartokinase is SEQ ID NO: 2 in which the L-serine in position 301 is replaced by a different proteogenic amino acid.

2. The replicatable nucleic acid of Claim 1, wherein the amino acid in position 301 is L-phenylalanine.

3. The replicatable nucleic acid of Claim 1, wherein the amino acid in position 301 is selected from the group consisting of L-aspartic acid, L-asparagine, L-threonine, L-serine, L-glutamic acid, L-glutamine, glycine, L-alanine, L-cysteine, L-valine, L-methionine, L-isoleucine, L-leucine, L-tyrosine, L-histidine, L-lysine, L-tryptophan, L-proline and L-arginine.

4. The replicatable nucleic acid of Claim 1, which is SEQ ID NO: 3.

5. The replicatable nucleic acid of Claim 1, which has the nucleotide sequence of SEQ ID NO: 1 in which the nucleotides at positions 901-903 are replaced with different nucleotides which encode a proteogenic amino acid other than L-serine.

6. The replicatable nucleic acid of Claim 5, wherein the nucleotide at position 902 is T.

7. The replicatable nucleic acid of Claim 1, which is a DNA.

8. A vector which contains the replicatable nucleic acid of Claim 1.

9. The vector of Claim 7, which is a plasmid.

10. The vector of Claim 8, which is replicable in corynebacteria.

11. Corynebacteria transformed with the vector of Claim 8.

12. Corynebacteria which contain the nucleotide sequence of Claim 1.

13. The Corynebacteria of Claim 12, which overexpress the aspartokinase.

14. A process for the preparation of L-lysine or L-lysine-containing animal feed additives, comprising:

a) fermenting corynebacteria in which alleles of the endogenous lysC gene are overexpressed under conditions suitable for forming the lysC gene product aspartokinase; and

b) isolating L-lysine or L-lysine-containing animal feed additive from the fermentation broth, wherein the corynebacteria produce L-lysine during the fermentation.

15. The process of Claim 14, wherein in the amino acid sequence belonging to the endogenous nucleotide sequence coding for the enzyme aspartokinase, the L-serine at position 301 is replaced by another proteogenic amino acid.

16. The process of Claim 15, wherein said another proteogenic amino acid is L-phenylalanine.

17. A process for the preparation of L-lysine or L-lysine-containing animal feed additives, comprising:

a) fermenting corynebacteria containing an endogenous nucleotide sequences coding for the enzyme aspartokinase, the L-serine in position 301 of the corresponding amino acid sequences being replaced by another proteogenic amino acid,

b) enriching the L-lysine in the fermentation broth, and

d) isolating the L-lysine or L-lysine-containing animal feed additive from the fermentation broth, optionally with constituents of the fermentation broth and/or the biomass.

18. A process for producing L-lysine, comprising fermenting the Corynebacteria of Claim 11 in a fermentation broth under conditions that the Corynebacteria produce L-lysine, and isolating the L-lysine.

19. A process for producing L-lysine, comprising fermenting in the Corynebacteria of Claim 12 in a fermentation broth under conditions that the Corynebacteria produce L-lysine, and isolating the L-lysine.

ABSTRACT

Alleles of the lysC gene from corynebacteria that code for desensitized aspartokinases, and to processes for the preparation of L-lysine using bacteria containing these alleles.

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Figure 1: Map of plasmid pK18mobsacB_lysC_S301F